In vivo global isotope metabolomics implicates the arginase pathway in ischemic retinopathy

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Proliferative diabetic retinopathy (PDR) is the most severe form of diabetic retinopathy and, along with diabetic macular edema, is responsible for the majority of blindness in adults. Therapeutic strategies for PDR are ineffective at curtailing disease progression; however a deeper understanding of the ocular metabolic landscape in PDR through metabolomic analysis may offer new therapeutic targets. Here, both global mass spectrometry-based metabolomics and global isotope metabolomic analysis were used to investigate metabolism. Initial analyses on vitreous humor from patients with PDR (n=7) and non-diabetic controls (n=10) revealed an upregulation of arginine and acylcarnitine metabolism in PDR. The oxygen-induced-retinopathy (OIR) mouse model, which exhibits comparable pathological manifestations to human PDR, revealed similar upregulation of arginine and other metabolites in the urea cycle, as well as downregulation of purine metabolism. We validated our findings by targeted multiple reaction monitoring and through the analysis of a second set of patient samples (PDR (n=10) and non-diabetic controls (n=17)). These results confirmed the consistent upregulation of arginine, proline, citrulline, ornithine and octanoylcarnitine in both the OIR mouse model and patient PDR samples. Global isotope metabolomics was used to investigate the predominance of arginine in PDR. The metabolic fate of U-^{15}N-arginine was determined in the OIR model, revealing a predominance of the arginase pathway to form proline, with no evidence of nitric oxide synthase

involvement or polyamine production. These results indicate that in PDR, the arginase pathway predominates, decreasing the availability of arginine for nitric oxide synthesis, a metabolite required for adequate endothelial cell function.